b.) Remarks

Claims 1-3 and 5-7 have been amended in order to recite the present invention with the specificity required by statute. No new matter has been added.

Claims 1-3 and 5-15 stand rejected under 35 USC 112, second paragraph as failing to particularly point out and distinctly claim the subject matter of the present invention since the Examiner is uncertain what the "or a DNA which hybridizes" language refers to. Additionally, the Examiner is also uncertain to which polypeptide "wherein 1 to 20 amino acids are deleted" refers. Accordingly, in response, claim 1 has been amended in order to address both of the Examiner's concerns.

Claims 1-3 and 5-15 stand rejected under 35 USC 112, first paragraph, as containing subject matter that was not described in the specification so as to reasonably convey to one of ordinary skill the inventors had possession of the claimed invention at the time the application was filed, and also for failing to reasonably enable one of ordinary skill to practice the claimed invention. The Examiner's bases for these rejections are set forth at pages 3-10 of the Office Action.

According to the Examiner, the claims are drawn to a microorganism expressing a polynucleotide genus that is only "approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO:3 encoding a protein having NADH dehydrogenase activity". The Examiner further states there is no structure-function correlation with regards to the members of this genus.

As to the Examiner's 69.8% calculation of sequence identity, such is the identity value of whole sequence. However, those of ordinary skill readily appreciate that

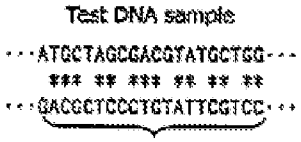
the whole DNA is not used as a hybridization probe. To the contrary, PCR primers or probes of short DNA such as oligonucleotides having 15-20 nucleoticles are used (see Molecular Cloning, third ed.) since the relative activity of oligonucleotides probe is higher than a whole DNA probe. Therefore, it is understood that the claimed DNA which has high (70%) identity over whole sequence of the probe DNA will have *extensive partial regions* having 100% identity with the oligonucleotide probes.

Moreover, when the identity of the overall sequence of test sample and a 20 nucleotide probe DNA is 70%[1], the 20 nucleotide oligonucleotide probe will not hybridize with the DNA test sample, because the permissible mismatching for hybridization between the probe and test sample is 5.2% (70.2 less 65) and so the number of permissible mismatched nucleotides is only a single nucleotide. On the other hand, it is understood that when 21 continuous nucleotides of a 30 nucleotide test DNA sample are identical with the oligonucleotide probe consisting of 21 nucleotides, the probe DNA will certainly hybridize with the DNA test sample.

This is illustrated below, for the Examiner's convenience.

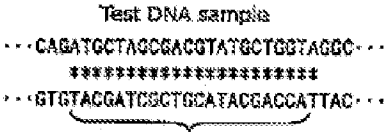
[1] When, for instance, 14 nucleotides within continuous 20 nucleotide of the partial region of DNA test sample are identical with the oligonucleotide probe containing 20 nucleotides

(1) 14/20 70% identity (whole DNA)



* The probe DNA does not hybridize with the test DNA sample

(2) 21/30 70% identity (whole DNA) but partially 100% identity



* The probe DNA does hybridize with the test DNA sample

That is to say, then, contrary to the Examiner's analysis, DNA that has only 69.8% identity with SEQ ID NO:3 will only hybridize when it has extensive DNA regions with approximately 100% identity to locally-existing sequences.

Thus, when the person skill in the art undertakes hybridization according to his or her routine knowledge, DNAs which have 69.8 % identity with SEQ ID NO:3, but do not have NADH dehydrogenase activity would not be detected.[2]

Claims 1-3 and 5-15 stand rejected under 35 USC 103 being obvious over Bott, Molenaar, Hollander and Nakagawa, for the reasons of record. The Examiner's bases in support of this rejection are set forth at pages 10-14 of the Office Action. Specifically, the Examiner states Bott shows that producing amino acids in *Corynebacterium glutamicum* involves NADH dehydrogenase, Molenaar teaches a type II NADH dehydrogenase identical to Applicants' SEQ ID NO:3 and also "that *Corynebacterium glutamicum* only express type II NADH dehydragenase (page 6887, right column 1st

[2] Moreover, the level of experimentation required by the foregoing is plainly routine to those of ordinary skill in this art.

paragraph)." Additionally, Hollandar teaches production of NADH and Nakagawa shows producing desired materials by fermenting *Escherichia coli*.

Initially, as to the foregoing, the Examiner states claims 5 and 7 are included in the rejection because "these claims are interpreted openly and not limited to the specific plasmid/*E. coli* strain." Such rejection is not well-understood. Clarification is respectfully requested.

Applicants previously distinguished the present invention from the prior art by pointing out there was no reason to arbitrarily select type-II NADH dehydrogenase to improve amino acid production because such is not at all limiting; *C. glutamicum* contains many other enzymes that consume NADH and NADPH. Moreover, Applicants further explained the art taught away from the claimed subject matter because Nakai explicitly teaches inactivating NADH-II does not affect amino acid production in *E. coli*.

In response, the Examiner now asserts at pages 14-16 that Nakai's showings in *E. coli* are immaterial because (i) *E. coli* NADPH dehydrogenases are unsimilar to the *C. glutamicum* NADH II dehydrogenase, and (ii) C. glutamicum has only a type II NADH dehydrogenase. These points are, therefore, addressed in turn below.

First, respectfully submitted, the Examiner's statement that *E. coli* NADH-II dehydrogenase is dissimilar to *C. glutamicum* NADH-II dehydrogenase because the sequence homology score between them is only 27% is incorrect. As is well-understood, the name of an enzyme is determined based on its function. In that regard, *C. glutamicum* NADH-II dehydrogenase plays the same role with the same function as *E. coli* NADH-II dehydrogenase. Indeed, Nakai does not opine or suggest that *C. glutamicum* NADH-II dehydrogenase is different from *E. coli* NADH-II dehydrogenase -- Nakai teaches that *E.*

coli has two kinds of terminal oxidases in its respiratory chain, named NDH-I (which has high energy efficiency) and NDH-II (which has low energy efficiency). *C. glutamicum* also has two of the same kinds of terminal oxidases, one of which is an electron transfer pathway of high energy efficiency (SoxM type oxidase) and the other of which is an electron transfer pathway of low energy efficiency (cytochrome bd type oxidase).[3]

Nakai further teaches that *E. coli* efficiently produce and accumulate amino acids when the high energy efficiency respiratory chain pathway is enhanced and the low energy efficiency respiratory chain pathway is deficient.

Therefore, it is indisputably established on the record that (I) *C. glutamicum* has a respiratory chain pathway of high energy efficiency (SoxM type oxidase) in which the oxidase plays a role of a primary dehydrogenase linked with central metabolism and (II) *C. glutamicum* has a respiratory chain pathway of low energy efficiency (cytochrome bd type oxidase) which is not involved in production of amino acids.

NADH-II of SEQ ID NO:4 described in the present invention is the respiratory chain pathway of low energy efficiency, therefore NADH-II of SEQ ID NO:4 has the same function as NDH-II of *E. coli* as described in Nakai, e.g., which is apparently not involved in amino acid production.

Because Nakai discloses that it is optimal to be deficient of NADH-II, the same respiratory chain pathway of low energy efficiency for producing amino acids, the present invention (in which amino acid production is improved by culturing *C. glutamicum* with amplified DNA encoding NADH-II), is necessarily unobvious over the prior art.

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[3] See [0005] and [0006] of Nakai.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

Claims 1-3 and 5-15 remain presented for continued prosecution.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

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